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DETERMINATION OF SE(IV), CD(II) AND PB(II) IONS IN HOMEOPATHIC DRUGS BY INVERSION VOLTAMMETRY METHOD

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Abstract: The conditions for identification and quantification of Se(IV), Cd(II) and Pb(II) ions in homeopathic drugs by inversion voltammetry method with the use of EAGRAPH software were established. The studies proved that the method was of high sensitivity in established conditions. The detection limits were 0.66 µg/mL, 0.08 µg/mL and 0.12 µg/mL for Se(IV), Pb(II) and Cd(II) ions, respectively. This method was characterized by repeatability of measurements, a wide range of linearity and satisfactory percent recovery.

Keywords: selenium, stripping voltammetry, homeopathic drugs

Selenium, similarly as manganese, chromium, zinc and cobalt, belongs to trace elements, which presence is indispensable for normal functioning of the organism. Selenium has a special role and therefore is sometimes called an element of life. This description concerns the role of selenium in the human organism: participation in processes of lipid peroxidation, DNA and RNA synthesis and detoxication of free radicals and peroxides (1, 2).

Deficiency as well as surplus of selenium may result in many disorders of circulatory system and skeleton (2). There are published data that demonstrate relationship between deficiency of selenium and neoplastic diseases (3). The key function of this element is ascribed to disorders in functioning of a selenium-dependent glutathione peroxidase, which results in elevated number of reactive oxygen species in human organism (4).

Selenium compounds have various effects on organism, because of dose-dependent action and therefore there is a need to search the methods which will be useful for selenium determination. Analytical methods have to be very sensitive and selective because of relatively low concentration of selenium as well as its occurrence together with other components in various complex drugs.

In the literature, proven voltammetric methods have been used to determine the selenium in materials of different origin (4–8, 9). The simultaneous determination of metal ions in environmental matrix composite used two methods, DPCSV (Differential Pulse Cathodic Stripping Voltammetry) for ions As(III) and Se(IV) and Cu(II) ions in differential pulse anodic stripping voltammetry (DPASV) for Pb(II), Cd(II) and Zn(II). After the initial mineralization, studies were conducted in ammonium buffer (pH = 9.30). The standard additions method was applied for determination of respective metal ions (6).

For the determination of selenium next to the lead in samples of milk, DPCSV and DPASV were used. Research were carried out using 0.1 M HCl solution and HMDE (Hanging Mercury Drop Electrode) as the working electrode. Under the conditions described, the peak potentials of the ions Se (IV) and Pb (II) were as follows: E = -0.56 V and E = -0.35 V. The results obtained were between 21.5 and 69.4 µg /L Se(IV) and 22.1 and 59.2 µg/L Pb(II), respectively. The study was preceded by the digestion solution of HNO_3:$HClO_4$ (1:1, v/v) (5).

Cathodic stripping voltammetry has been applied for the determination of selenium ions in the garlic. The linear range of the calibration curve reached from the detection limit up to 2.0×10^{-8} to 6.0×10^{-9} M for Se(IV), respectively. The correlation coefficients for all analyzes ranged from 0.9993 to 0.9997 s (9).

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For determinations of selenium the following techniques were used: atomic spectrometry (10–12), gas chromatography in combination with mass detection (13), as well as the electrophoretic method (14).

As homeopathic medicines are widely used in therapy, it is justified to undertake studies aimed at both their standardizations in respect to determinations of active ingredients, as well as evaluation of their purity, mainly the presence of toxic metals impurities such as cadmium and lead (15).

In the present study, the direct determination of Se(IV) ions in the formulations such as homeopathic drops and injections, and pollution by Pb(II) and Cd(II), which may originate from the raw materials used to manufacture these drugs, was performed.

In order to achieve this goal, DPASV method with computer software EAGRAPH was developed. In searched literature, a lack of data on the determination of Se(IV) beside the Cd(II) and Pb(II) in the homeopathic preparations employing the DPASV method justified the undertaken studies.

The content of the inorganic elements was determined in the biological materials. The samples of urine were analyzed for the three elements Pb(II), Cd(II) and Ni(II) by DPASV and HNO_3 0.1 M as supporting electrolyte. Urine samples were diluted with deionized water and measured by using graphite furnace atomic absorption spectrometry (GFAAS). The results showed that GFAAS can be an effective tool for profiling, analysis of many different elements and speciation of biological samples (17).

EXPERIMENTAL

Solutions and preparations

Standard solutions of Se(IV), Pb(II) and Cd(II) at a concentration of 1.0 mg/mL were prepared for studies by diluting with water to obtain the final concentration of 0.1 mg/mL.

A basic electrolyte was ammonium buffer (pH = 10.00); 2 mL 20% NH_3 aq. and 5.35 g NH_4Cl, filled with deionized water to 1 L volume. A

Table 1. Results of validation parameters of Cd(II), Pb(II) and Se(IV) ions.

Parameter		Se(IV)	Cd(II)	Pb(II)
Basic electrolyte		Ammonium buffer		
Peak potential [mV]		–864	–635	–575
Linearity range [µg/mL]		y = 0.216x + 0.408 r = 0.9993	y = 0.020x + 0.110 r = 1.0000	y = 0.015x + 0.229 r = 0.9997
LOD [µg/mL]		0.66	0.12	0.08
LOQ [µg/mL]		1.99	0.37	0.24
Accuracy [%] n = 6	80%	$X_{śr}$ = 100.7% SD = 1.129 $S_{Xśr}$ = 0.461 RSD = 1.10%	$X_{śr}$ = 99.99% SD = 1.34 $S_{Xśr}$ = 0.423 RSD = 1.30%	$X_{śr}$ = 100.2% SD = 0.915 $S_{Xśr}$ = 0.374 RSD = 1.00%
	100%	$X_{śr}$ = 98.48% SD = 0.705 $S_{Xśr}$ = 0.315 RSD = 0.70%	$X_{śr}$ = 100.3% SD = 0.779 $S_{Xśr}$ = 0.318 RSD = 0.80%	$X_{śr}$ = 101.6% SD = 0.626 $S_{Xśr}$ = 0.256 RSD = 0.63%
	120%	$X_{śr}$ = 102.9% SD = 1.003 $S_{Xśr}$ = 0.410 RSD = 1.00%	$X_{śr}$ = 100.0% SD = 0.616 $S_{Xśr}$ = 0.251 RSD = 0.61%	$X_{śr}$ = 100.1% SD = 0.584 $S_{Xśr}$ = 0.261 RSD = 0.60%
Mean intra-day precision n = 6		$x_{śr}$ = 4.90 SD = 0.498 $S_{Xśr}$ = 0.203 RSD = 0.60%	$x_{śr}$ = 1.69 SD = 0.006 $S_{Xśr}$ = 0.0003 RSD = 3.20%	$x_{śr}$ = 4.90 SD = 1.111 $S_{Xśr}$ = 0.454 RSD = 4.80%
Mean inter-day precision n = 6		$x_{śr}$= 4.95 S_D = 0.006 $S_{Xśr}$ = 0.002 RSD = 1.99%	$x_{śr}$ = 1.70 S_D = 0.483 $S_{Xśr}$ = 0.199 RSD = 0.73%	$x_{śr}$ = 4.99 S_D = 0.006 $S_{Xśr}$ = 0.003 RSD = 2.00%

required pH was obtained by titration with 1 M ammonia solution.

Homeopathic preparations were: Selenium Hommacord (ampoules), Selenium Hommacord (drops), Selenium (ampoules) and Selenium (drops) all from Heel, Baden-Baden, Germany.

Reagents

NH_3 aq. solution (pure > 99%), NH_4Cl (pure > 99%) and H_2SeO_3 (pure > 99%) were from POCH, Gliwice, Poland. Double-distilled water was from Millipore (Germany) and nitrogen of purity class 5.0 was obtained from Linde Gas, Poland.

Instrumentation

Ea9 multipurpose voltammeter designed for polarographic and voltammetric analyses, with Eagraph software (developed by MTM – ANKO, Poland) was used. The following were the electrodes: controlled growth mercury drop electrode (CGMDE); reference: silver-silver chloride electrode (Ag/AgCl DB), and auxiliary: platinium wire electrode all from MINERAL (Warszawa, Poland).

Procedure

The measurements were performed by inversion voltammetry using DPASV technique of pulse amplitude of 20 mV and a quite time of 20 ms. The ions under study were concentrated for 180 s at potential of –1400 mV. Prior to the measurement, the voltammetric cell vial was filled with corresponding solution at composition of 1.0 mL standard solution at concentration from 0.5 µg/mL to 8.5 µg/mL or 1.0 mL studied solution, 6.3 mL ammonium buffer (pH 10.00), and filled in with double-distilled water to the volume of 25 mL. Prior to measurement, deoxygenation was performed by passing nitrogen for 5 min.

The voltammetric curve was recorded by making three measurements for each concentration and taking average of these measurements.

Method validation

The method validation was performed according to ICH requirements (16). Therefore, linearity, limit of detection, and limit of quantification, accuracy, precision, and intermediate precision were established (Table 1).

Specificity

Comparison of potentials values of metal ions determined for standard solutions with studied solutions in tested conditions was used to evaluate specificity of the method. The studies were performed for basic electrolyte without ions and in the presence of each ion individually or in their mixture. As a criterion of acceptance, position of peaks recorded on voltammograms and values of current intensity were assumed.

Linearity

The appropriate Se (IV), Cd(II) and Pb(II) standard solutions in the amounts 5, 25, 45, 65 and 85 µL were measured and placed in 10.0 mL volumetric flasks. Then, buffer solution was added into each flask to the volume mentioned above. The 1.0 mL volume of prepared solutions was taken for determination.

Limit of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the corresponding curves, using slopes of a straight line (a) and standard error of slope in linear regression (S_y), and the following formulas were employed:

$$LOD = 3.3 \times S_y/a \text{ and } LOQ = 10\times S_y/a$$

Accuracy

The accuracy was evaluated based on the percent of recovery. In computing the concentrations of each metal ion compared to the amount added to the Selenium Hommacord preparation whose ion concentrations had been earlier determined prior to adding standard solutions, were taken into account. The accuracy was estimated for three levels of concentrations 80, 100, and 120%.

Precision

The precision was evaluated from 6 measurements for a reference sample containing 5.00 µg/mL Se(IV), 2,50 µg/mL Cd(II) and 2,50 µg/mL Pb(II).

Intermediate precision

The intermediate precision was evaluated similarly to precision evaluation. The analysis was performed after 24 h and the same solutions and instrument were used.

RESULTS AND DISCUSSION

Based on the results obtained of the determination of ion content test plan, the first time defined concentration ranges studied metal ions Se(IV), declared in the composition of homeopathic preparations as active ingredients, and ions of Pb (II) and Cd (II) pollutants.

Table 2. Results of determination of concentration of Se(IV). Cd(II) and Pb(II) ions in homeopathic preparations.

Content	Se (IV) [µg/mL]	Cd(II) [µg/mL]	Pb(II) [µg/mL]
SELENIUM HOMMACORD (injections)	0.86; 0.90.; 0.92 0.93; 0.98; 0.99 $X_{śr}$ = 0.93 SD = 0.075 RSD = 3.98%	1.40; 1.40; 1.40 1.40; 1.45; 1.45 Xśr. = 1.42 SD = 0.026 RSD = 1.80%	–
SELENIUM HOMMACORD (drops)	1.00; 1.10; 1.13 1.15; 1.15; 1.25 Xśr = 1.13 SD = 0.077 RSD=6.88%	–	0.69; 0.69; 0.69 0.71; 0.71. 0.71 Xśr = 0.70 SD = 0.016 RSD = 1.82%
Selenium (injections)	0.81; 0.82; 0.82 0.82; 0.82; 0.83 Xśr = 0.82 SD = 0.011 RSD = 1.34%	2.00; 2.05; 2.05 2.05; 2.05; 2.I5 Xśr = 2.06 SD = 0.058 RSD = 2.00%	–
Selenium (drops)	5.94; 5.95. 5.95 6.32; 6.32; 6.32 Xśr = 6.16 SD = 0.052 RSD = 1.90%	–	–

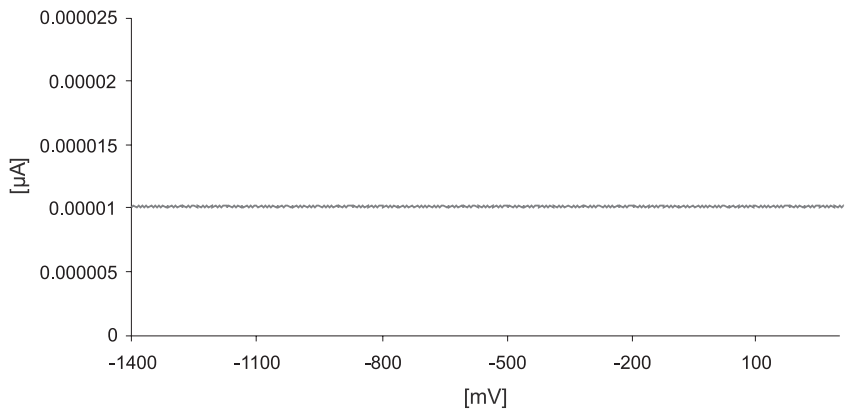

Figure 1. Voltammogram of ammonia buffer (pH = 10.00) recorded by DPASV method

The results of the studies have shown that the developed DPASV method allow direct determination of Se(IV), Cd(II), Pb(II) that occur next to each other in homeopathic preparations.

Solution of ammonium buffer in the range of potentials from –1400 mV to +250 mV did not give any change of current intensity that may have impact on determination of studied ions (Fig. 1). Peaks recorded on voltammograms differed with potential values of –864 mV, – 635 mV and –575 mV for Se(IV), Cd(II) and Pb(II), respectively (Fig. 2).

The method is characterized by a wide range of linearity in the test concentration ranges from 0.5 to 8.5 ng/mL. Curves are straight lines while maintaining the good correlation coefficients between 0.9993 and 1.00000. The sensitivity of the methods specified for the individual components is high. LOD is situated between 0.08 ng/mL and 0.66 ng/mL and LOQ is from 0.24 ng/mL to 1.99 ng/mL. Precision

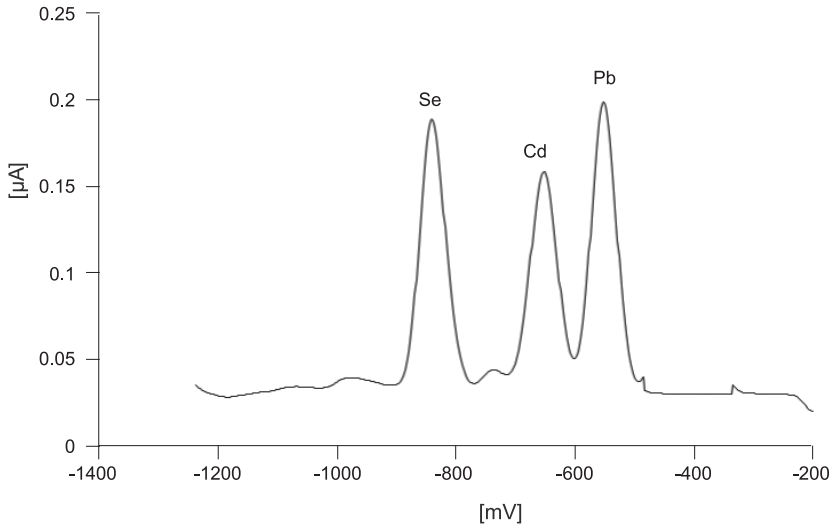

Figure 2. Voltammograms of Se(IV), Cd(II) and Pb(II) ions recorded by DPASV method.

determined on the basis of recovery as the mean of 80, 100 and 120% is from 98.48% to 102.90%. Precision of repeatability of results of the method is characterized by narrow confidence interval and the values of the RSD range from 0.60 to 4.80%. Good precision was also observed when determining the precision of intermediate performed after 24 h. RSD falls within the range 0.73–2.00%.

Detailed results are presented in Table 1.

The results of determination of average concentration of Se(IV) in three homeopathic preparations were in the concentration range from 0.82 to 1.13 µg/mL, whereas in one preparation this concentration was definitely higher, 6.16 µg/mL.

The level of Cd(II) and Pb(II) ions established in µg/mL did not exceed acceptable pharmacopoeial limits for impurities according to European Pharmacopoeia (Table 2).

Developed method is widely available and relatively cheap in comparison to the spectroscopic methods in respect of which it may be an alternative.

CONCLUSION

Developed method is useful for simultaneous DPASV identification and direct determination of Se (IV), Cd(II) and Pb(II) in the preparation of homeopathic formulations.

In the conditions set out in the analysis (ammonium buffer pH = 10.00), there are no interference currents next of ingredients, which may be of metal ions or matrix derived from drugs.

The sensitivity of the presented voltammetric methods is high and allows to make the determinations at concentrations from 0.70 to 6.16 ng /mL for individual ions, directly, without the need of their evolution.

The validation method presented meets the criteria for the methods used in the analysis of trace analysis of metals ions.

Based on the results of the determinations, there were established concentration levels for individual ions that occur in a randomly selected group of homeopathic products.

The content of the individual ions does not exceed the dose used in therapy for medicinal products containing ingredients tested and there is no danger of the use in doggy treat homeopathic preparations from a toxicological point of view.

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